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ORIGINAL ARTICLE

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Epidermal growth factor receptor is a marker for syncytiotrophoblastic cells in testicular germ cell tumors

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Abstract The epidermal growth factor receptor (EGFR) has been implicated in the pathogenesis, therapy and prognosis of various tumor types. The aim of this study was to investigate EGFR expression in a large series of testicular germ cell tumors (TGCTs). A total of 88 TGCTs (37 of pure type and 51 of mixed type) comprising a total of 44 seminoma, 49 embryonal carcinoma, 32 yolk sac tumor, 28 teratoma and 7 choriocarcinoma components were immunostained for EGFR. EGFR reactivity was observed in the stromal cells of embryonal carcinoma (29%) and in epithelial compartments of teratoma (71%). In addition, EGFR staining was consistently detected in syncytiotrophoblastic cells of choriocarcinoma, seminoma, embryonal carcinoma and yolk sac tumor components. EGFR staining, similar to β-human chorionic gonadotropin (HCG) immunohistochemistry, was efficiently able to identify syncytiotrophoblastic cells in TGCTs. This study shows that EGFR is expressed in a subset of testicular germ cell tumors and suggests that EGFR may be a useful marker for syncytiotrophoblastic cells.

Keywords Epidermal growth factor receptor (EGFR) · Testicular germ cell tumors · Syncytiotrophoblastic cells

Introduction

The epidermal growth factor receptor (EGFR) is a 170-kDa growth factor receptor tyrosine kinase [20]. Upon binding of its cognate ligands, which include EGF and transforming growth factor alpha (TGFα), it activates signal transduction cascades that contribute to the control of cell differentiation, proliferation, apoptosis, migration and adhesion [25]. The EGFR protein is frequently overexpressed in various types of cancer, and its overexpression has been correlated with poor prognosis in head and neck, ovarian, cervical, bladder and esophageal cancer [17, 19]. Novel treatment modalities that specifically inhibit EGFR-mediated signal transduction have recently become available [14]. This development provides a rationale for the systematic identification of tumor types that overexpress EGFR and therefore represent potential candidates for EGFR-targeted therapy.

Testicular germ cell tumors (TGCTs) are the most common type of cancer in young adult males. TGCTs can be classified as seminomas and non-seminomatous germ cell tumors, the latter group comprising embryonal carcinoma, yolk sac tumor, polyembryona, teratoma and chroriocarcinoma [6]. TGCTs occur either as tumors with one homogeneous histological pattern (pure types) or show more than one histological pattern (mixed types). The various histological forms of TGCT are clinically and biologically diverse despite sharing intratubular malignant germ cells as a common precursor [2, 3, 5]. Multiagent cisplatin-based chemotherapy has significantly improved the prognosis of patients with advanced TGCT. However, disease recurrence and progression still occur, indicating the need for additional treatment modalities [2].

The purpose of the present study was to analyze the expression of the EGFR protein in a large series of TGCT using immunohistochemistry.

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Materials and methods

A total of 182 TGCTs were retrieved from the surgical pathology files of the Department of Pathology, University of Zürich, covering a period of 9 years (1993 to 2001). Tumors were classified according to the World Health Organization (WHO) classification of testis tumors [16]. Eighty-eight TGCTs were selected for EGFR immunohistochemistry. This group consisted of 37 pure-type and 51 mixed-type TGCTs comprising a total of 44 seminoma, 49 embryonal carcinoma, 32 yolk sac tumor, 28

Table 1 Epidermal growth factor receptor (EGFR) expression in testicular germ cell tumors according to histological tumor type

Histological tumor type	Number of tumor components	EGFR reactivity
Seminoma	44	0[a]
Embryonal carcinoma	49	14 (29%)[a]
Yolk sac tumor	32	0[a]
Teratoma	28	20 (71%)
Choriocarcinoma	7	7 (100%)

[a] EGFR expression was observed in syncytiotrophoblastic cells of 5 seminoma, 20 embryonal carcinoma and 10 yolk sac tumor components

teratoma and 7 choriocarcinoma components (Table 1). Immunostaining was performed on one to four representative tissue blocks for each tumor. Three-micron-thick sections of formalin-fixed, paraffin-embedded tissue samples were mounted on glass slides (SuperFrost Plus; Menzel, Braunschweig, Germany), dried for 20 min at 59°C, deparaffinized and rehydrated. Prior to incubation with primary antibody (EGFR, clone 31G7, dilution 1:30; Zymed Laboratories, San Francisco, USA), antigen retrieval was performed with the Ventana protease 1 reagent (Ventana Medical Systems, Tucson, Arizona, USA) for 2 min. Pretreatment of sections, antibody incubation and detection of primary antibody (Ventana Enhanced Alkaline Phosphatase Red Detection Kit) were performed on a Benchmark immunohistochemistry staining system (Ventana Medical Systems). EGFR immunoreactivity was scored separately for each histological component. Epidermis of normal human skin was used as a positive control, and primary antibody was omitted in negative controls.

For β-human chorionic gonadotropin (β-HCG) staining, antigen retrieval was performed with Ventana cell conditioning solution for 36 min at 100°C prior to incubation with primary antibody (β-HCG, clone h6, dilution 1:20.000; BMA Biomedicals, Augst, Switzerland). Pretreatment of sections, antibody incubation and detection of primary antibody (Ventana Basic DAB Detection Kit) were performed on a Benchmark immunohistochemistry staining system.

Results

EGFR staining was comparable in pure and mixed-type TGCTs. A strong membranous EGFR immunoreactivity was found in syncytiotrophoblastic cells of choriocarcinomas (100%; Fig. 1A). EGFR expression was also detected in the majority of teratomas (71%). Staining was found in squamous and columnar epithelia with predominant labeling of the basal cell layers (Fig. 1B, C), whereas cartilage and other non-epithelial (e.g. neural) tissues remained unstained. In 29% of embryonal carcinomas, EGFR immunostaining was observed in the stromal tumor cell compartment (Fig. 1D). EGFR immunoreactivity was absent in seminoma and yolk sac tumor cells. No EGFR staining was observed in intratubular malignant germ cells.

To study a potential role for EGFR as a marker for syncytiotrophoblastic cells, adjacent sections of 38 tumor samples with syncytiotrophoblasts were stained for EGFR and β-HCG [9]. In addtion to choriocarcinoma, syncytiotrophoblastic cells were found in 5 seminoma, 20 embryonal carcinoma and 10 yolk sac tumor components. In all tumors (100%), syncytiotrophoblastic cells identified by β-HCG immunohistochemistry also showed strong EGFR staining (Fig. 1E).

Discussion

In this study, we demonstrated EGFR expression in a subset of TGCTs, namely in the stromal compartment of embryonal carcinomas and in the squamous and columnar epithelia of teratomas. EGFR staining in the basal layers of squamous and columnar epithelia is consistent with the physiological functions of the EGFR in the proliferation and differentiation control of epithelial cells [25]. EGFR expression was also found in syncytiotrophoblastic cells, either as components of choriocarcinoma or scattered as single cells in other types of TGCT. This expression pattern corresponds to previous observations that EGFR is expressed in the implantation trophoblast, in the trophoblast of normal placenta and in gestational trophoblastic diseases [1, 7, 10, 11, 22].

TGCTs are often associated with elevated serum HCG levels, which can be due to the presence of either choriocarcinoma or single syncytiotrophoblasts. Differentiation between these possibilities is clinically important. Identification of choriocarcinoma in a mixed TGCT is associated with poor prognosis, whereas scattered syncytiotrophoblastic cells appear to exert no adverse effects on survival [21, 23, 24]. Immunohistochemical characterization of syncytiotrophoblastic cells has revealed expression of β-HCG [9], pregnancy-specific beta-1-glycoprotein [12], human placental lactogen [12], inhibin [13], epithelial membrane antigen [18], and cytokeratins 7, 8, 18 and 19 [4, 6]. Of these, β-HCG has been considered to be the most valuable marker for syncytiotrophoblastic cells. We found that EGFR immunolabelling was able to detect syncytiotrophoblasts with an efficiency comparable to that of β-HCG staining. This result suggests that the EGFR can be used as a marker for syncytiotrophoblastic cells instead of β-HCG or to resolve an equivocal β-HCG staining.

Two recent studies have also used immunohistochemistry to investigate EGFR expression in TGCTs [8, 15]. Our observation of EGFR expression in choriocarcinoma and single syncytiotrophoblastic cells confirms these previous results [8, 15]. In addition, we also found EGFR expression in embryonal carcinomas and teratomas, which had not been reported previously. These differences are most likely due to the larger size of our TGCT series and the more detailed histological analysis in our study.

Novel treatment modalities that specifically target EGFR signal transduction, such as anti-EGFR monoclonal antibodies and tyrosine kinase inhibitors, have

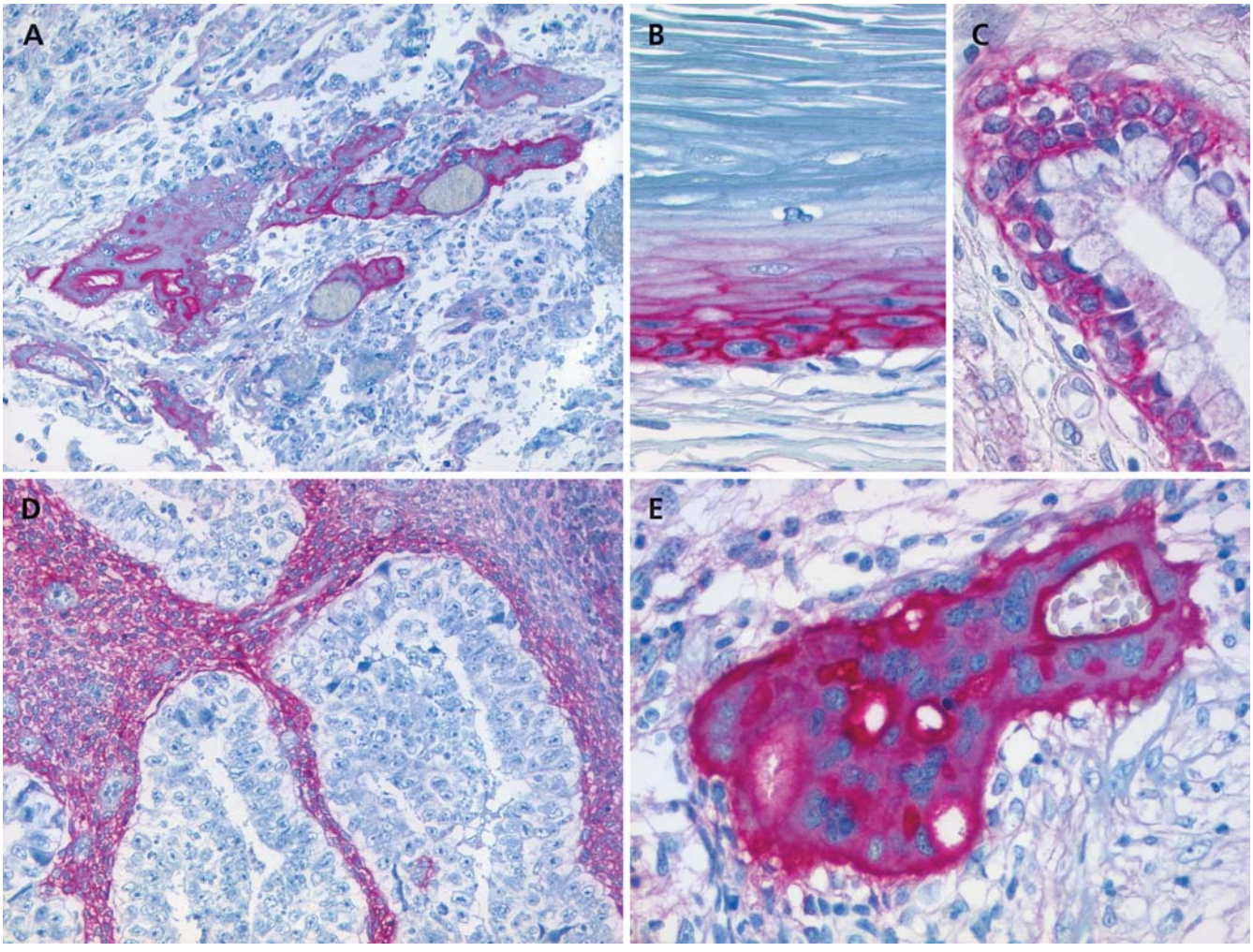

Fig. 1 Epidermal growth factor receptor (EGFR) expression in testicular germ cell tumors. EGFR immunoreactivity was detected in syncytiotrophoblastic cells of choriocarcinoma (**A**), squamous (**B**) and columnar (**C**) epithelia of teratoma, stromal cells of embryonal carcinoma (**D**) and in single syncytiotrophoblastic cells of teratoma (**E**)

recently become available [14]. Our result of a rather restricted EGFR expression pattern suggests that targeting EGFR signal transduction will be of limited value in the treatment of patients with TCGT.

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